# ASSOCIATION BETWEEN TWO COMMON POLYMORPHISMS OF VITAMIN D BINDING PROTEIN AND THE RISK OF CORONARY ARTERY DISEASE: A CASE-CONTROL STUDY

POVEZANOST IZMEĐU DVA ČESTA POLIMORFIZMA VITAMIN D-VEZUJUĆEG PROTEINA I RIZIKA OD KORONARNE ARTERIJSKE BOLESTI: ANAMNESTIČKA STUDIJA

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## Summary

**Background:** Coronary Artery Disease (CAD) is one of the most widespread non-communicable diseases. Vitamin D-binding protein (VDBP) and its genetic polymorphisms have been highlighted as the susceptible components for CAD. The aim of the present study was to examine the association of VDBP single nucleotide polymorphisms (SNPs) – rs7041 and rs4588 – with CAD susceptibility among the Iranian population.

**Methods:** A total of 143 men with CAD and 145 healthy age-sex matched controls underwent genotyping for the — rs7041 and rs4588 polymorphisms using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Serum level of 25(OH)D was assayed using microplate colorimetric enzyme immunoassay.

**Results:** We found a significant association between GG genotype (rs7041) and CAD (p=0.02, OR=0.537 95% CI =0.306–0.944). Regarding rs4588 polymorphism, a significant difference was observed in which the CA genotype

## Kratak sadržaj

**Uvod:** Koronarna arterijska bolest (CAD) jedno je od najrasprostranjenijih hroničnih oboljenja. Vitamin D-vezujući protein (VDBP) i njegovi genetski polimorfizmi predočeni su kao podložne komponente za CAD. Cilj ove studije bio je da se ispita povezanost između polimorfizama pojedinačnih nukleotida (SNPs) proteina VDBP –rs7041 i rs4588 i podložnosti CAD u populaciji Iranaca.

**Metode:** Ukupno 143 muškarca sa CAD i 145 zdravih kontrolnih ispitanika odgovarajućeg uzrasta i pola podvrgnuto je genotipizaciji za polimorfizme –rs7041 i rs4588 pomoću metode lančane reakcije polimeraze odnosno polimorfizama dužine restrikcionih fragmenata (PCR-RFLP). Nivo 25(OH)D u serumu određen je pomoću kolorimetrijskog enzimskog imunoeseja na mikroploči.

**Rezultati:** Otkrili smo značajnu povezanost između genotipa GG (rs7041) i CAD (p=0,02, OR=0,537 95% CI=0,306– 0,944). Što se tiče polimorfizma rs4588, uočena je značajna razlika, pri čemu su genotip CA (P=0,00032, OR=2,578,

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(p=0.00032, OR=2.578, 95% CI=1.579–4.208) and allele A (P=0.028, OR=1.491, 95% CI=1.043–2.132) were significantly higher in CAD patients compared to controls. In spite of lower serum levels of 25(OH)D in CAD patients, we found no significant association between these SNPs and Vitamin D serum concentrations.

**Conclusions:** We concluded that VDBP polymorphisms affect the susceptibility to CAD in Iranian men. Therefore, further studies are required to clarify the association of VDBP phenotypes and its serum levels with CAD.

**Keywords:** coronary artery disease, polymorphism, vitamin D-binding protein, 25(OH)D

95% CI=1,579–4,208) i alel A (P=0,028, OR=1,491, 95% CI=1,043–2,132) bili značajno viši kod obolelih od CAD u poređenju s kontrolom. Uprkos nižim serumskim nivoima 25(OH)D kod pacijenata sa CAD, nije otkrivena značajna povezanost između ovih polimorfizama i koncentracija vitamina D u serumu.

**Zaključak:** Zaključili smo da polimorfizmi VDBP utiču na podložnost CAD kod iranskih muškaraca. Dakle, potrebne su dalje studije kako bi se razjasnila povezanost između fenotipova VDBP i njegovih nivoa u serumu i CAD.

**Ključne reči:** koronarna arterijska bolest, polimorfizam, vitamin D-vezujući protein, 25(OH)D

## Introduction

Coronary artery disease (CAD) is one of the significant leading causes of morbidity and mortality in the general population around the world (1–4). Its incidence is rapidly increasing in developing countries such as Iran (5–8). In spite of sufficient treatments, patients have a considerable mortality rate (7). A number of meta-analyses on prospective and interventional studies have constantly indicated that low circulating 25-hydroxy vitamin D (25(OH)D) points to increased risk of cardiovascular disease and its consequent mortality (9–17). Studies also reported that the active form of vitamin D is absolutely involved in the regulation of the renin angiotensin aldosterone system and immune system (18).

The presence of the vitamin D receptor (VDR) in almost all organs including the heart has motivated researchers to study the role of this vitamin in the context of general and cardiovascular health (19–22). Recent studies have focused on the role of its transporter, vitamin D-binding protein (VDBP), which is well known as Gc-globulin. VDBP binds to considerable amounts of the biologically active metabolite $1,25(OH)_2D$ and also 25(OH)D (23). Moreover, VDBP participates in immune regulation, fatty acid carriage, cell differentiation and cell proliferation, apoptosis and anti-angiogenesis (24, 25).

In humans, the VDBP gene is mapped on chromosome 4q12-q13 and consists of 13 exons and 12 introns. This gene is extremely polymorphic, with three major variants and about 124 rare ones (26). Two common point mutations of SNPs, rs7041 and rs4588, found in exon 11, make up three major isoforms and protein products including GC1F (Asp416, Thr 420; T,C), GC1S (Glu 416, Thr 420; G,C), and GC2 (Asp416, Lys420; T,A) (24, 27). These SNPs have diverse ethnic and geographic distributions and also various levels of protein activity, which affect its vitamin D binding ability and response to vitamin D supplementation (25, 28).

There is evidence that decreased levels of VDBP are associated with low flow in coronary arteries, endothelial disruption and atherosclerosis in patients with normal coronary arteries on coronary angiography (29). Furthermore, reduction in circulating levels of VDBP in young post-MI patients was statistically linked with the number of involved coronary arteries (30).

Although several studies have investigated the associations of 25(OH)D with the risk of CAD, there is a limited number of studies on the associations between VDBP and incidence of CAD. This study was designed to explore the associations of two common VDBP polymorphisms, rs7041 and rs4588, with CAD susceptibility in the Iranian population.

## Materials and Methods

### Study population

This case-control study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Number: IR.SBMU.RAM.REC. 1394.192). The patients recruited in this study were a total of 145 men with the median (IQR) age of 56.8±9.9 that were diagnosed with three vessels CAD. The severity of CAD was determined based on the number of involved vessels with more than 50% stenosis. The patients were candidates for coronary artery bypass graft (CABG) referred to Tehran Heart Center, Tehran, Iran. The patients were under anti-hypertensive and cholesterol-lowering treatments, while Aspirin was discontinued at least one week befor CABG preparations.

Furthermore, the study included 145 sex-age matched healthy controls with the median (IQR) age of 55.2±14.2 with confirmed 5 to 10 percent stenosis in their coronary arteries referred to the mentioned clinics. The healthy controls were not using anti-hypertensive and cholesterol-lowering agents. All control subjects were selected after careful inspection by a cardiovascular specialist.

For all subjects, a complete medical history, with questions about smoking habits, history of hypertension and diabetes mellitus, was obtained for their clinical records.

The individuals with diabetes mellitus, malignancy, myocardial infarction, unstable angina, previ-

ous coronary intervention, inflammatory diseases and other chronic diseases were not included. Moreover, subjects receiving antioxidant therapy or vitamin D supplement in the previous 12 months, and smokers were not included in the study. Informed consent was obtained from all participants according to the Declaration of Helsinki and accepted codes of the university ethics committee.

### Measurements

Systolic and diastolic blood pressures were measured twice on the right arm of the subjects after resting for at least 10 min in a comfortable position (Omron, M6 Comfort HEM-7321-E, Japan). Body Mass Index (BMI) was calculated as weight in kilograms divided by the height in meters squared. The Waist Circumference was taken at the midpoint between the iliac crest and the lower rib margin.

Fasting blood samples were obtained from CAD patients between 6:00 and 7:00 AM into vacutainer tubes before CABG. Fasting blood samples of healthy controls were drawn after angiography. Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Triglyceride (TG), and Fasting Blood Glucose (FBG) were measured by an enzymatic colorimetric method using commercial kits (Pars Azmon Inc., Iran) on an auto-analyzer (Hitachi 917 Ltd, Tokyo, Japan). Calcium and phosphorus were measured using commercial kits (Griener Diagnostic, Bahlingen, Germany).

### VDBP Genotyping

DNA was isolated from peripheral blood leukocytes using a salting out method (31). VDBP genotypes of rs7041 and rs4588 polymorphisms were detected using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. The oligonucleotide primer sequences were obtained from (32) and were confirmed in NCBI (*Table I*). In each 0.20 mL PCR reaction tube, 1 µL of genomic DNA (~100 ng/mL), 1 µL of each primer (10 µmol/L), 10 µL of 2× Prime Taq Premix (ampliqon 2× master mix red), and 7 µL ddH$_2$O were added regarding the following protocol: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C

**Table I** Primer sequences of rs7041 and rs4588 polymorphisms.

| SNP primer sequences | |
|---|---|
| Forward | 5'AAATAATGAGCAAATGAAAGAAGAC 3' |
| Reverse | 5'CAATAACAGCAAAGAAATGAGTAGA 3' |

for 30 s, extension at 72 °C for 30 s min, and final extension at 72 °C for 5 min. The 483 bp PCR product was digested by relevant restriction enzymes. The DNA fragments 297 and 186 bp for rs7041 obtained by Hae III, and 305 and 178 bp fragments for rs4588 obtained by Sty I, were observed by electrophoresis on 2% agarose gel with red gel staining. Sequencing method was used for random PCR products to confirm the RFLP procedure.

### Vitamin D circulating levels

The serum levels of 25(OH)D were measured using a competitive ELISA kit (AccuBind® Monobind Inc. Lake Forest, CA 92630, USA, Product code: 7725-300), according to the protocol. The kit measured both 25(OH) D$_3$ and 25(OH) D$_2$. The sensitivity of the kit was 0.67 ng/mL and the within and between assay precision were 4.62% and 5.38%, respectively.

### Statistical analysis

Data were analyzed by the statistical software SPSS 20 (SPSS, Chicago, IL). Differences in genotypic and allelic frequencies between patients and controls were reported as percentages and odds ratio (OR) with 95% confidence intervals (CI) and were examined by Chi-Square ($\chi^2$) test. Quantitative variables were tested for normality by the Shapiro-Wilk test. The normally distributed variables were expressed as Mean ± SD and tested by t-test or ANOVA, where indicated. The continuous variables without normal distribution were displayed as Median (interquartile range) and tested by Mann-Whitney U or Kruskal-Wallis. P values of less than 0.05 were considered statistically significant. Tests for deviation from the Hardy-Weinberg equilibrium (HWE), haplotypes and linkage disequilibrium (LD) measures were obtained by SHEsis software (33, 34).

### Results

In the current study, 143 men with the median (IQR) age of 57 (53–63) with confirmed CAD and also 145 healthy controls with the median (IQR) age of 55 (49–64) underwent genotyping for two SNPs in the VDBP gene. Two cases were excluded due to missing clinical data.

There were no significant differences in median age between the affected patients and controls. The demographic and clinical characteristics and the results of genotype and allele frequencies are given in *Tables II* and *III* respectively. Allelic and genotypic frequencies of rs7041 and rs4588 polymorphisms were in the Hardy-Weinberg equilibrium (P>0.05).

The WC (P-value = 0.001), HDL-C (P-value < 0.001), LDL-C (P-value = 0.014), TC (P-value

**Table II** Anthropometric data and clinical characteristics of CAD patients and Control group.

| Characteristics | CAD | Healthy controls | Mann-Whitney U |
|---|---|---|---|
| | Median (IQR) | Median (IQR) | p-value |
| Age (yr) | 57 (53–63) | 55 (49–64) | 0.058 |
| BMI (kg/m$^2$) | 26 (24–29) | 26 (24–29) | 0.818 |
| WC (cm) | 98 (92–105) | 94 (88–97) | 0.001 |
| FBG (mmol/L) | 5.16 (4.77–5.49) | 5.05 (4.66–5.49) | 0.416 |
| LDL-C (mmol/L) | 2.56 (2.07–3.10) | 2.25 (1.83–2.84) | 0.014 |
| HDL-C (mmol/L) | 0.9 (0.75–1.01) | 1.03 (0.85–1.21) | <0.001 |
| TC (mmol/L) | 4.14 (3.60–4.97) | 3.60 (3.03–4.35) | <0.001 |
| TG (mmol/L) | 1.24 (0.94–1.75) | 1.51 (1.11–2.22) | 0.001 |
| Ca (mmol/L) | 2.25 (2.25–2.50) | 2.25 (2.25–2.25) | 0.842 |
| Phos (mmol/L) | 0.95 (0.81–1.13) | 1.01 (0.84–1.13) | 0.278 |
| 25(OH)D (nmol/L) | 25.38 (15.63–38.56) | 29.66 (20.65–51.88) | 0.018 |
| SBP (mmHg) | 120 (120–130) | 120 (110–120) | 0.048 |
| DBP (mmHg) | 80 (70–80) | 72 (70–80) | 0.041 |

Continuous variables are presented as median (interquartile range: P25–P75).
Differences between cases and controls were obtained based on the Mann-Whitney U test.

CAD, Coronary Artery Disease; BMI, Body Mass Index; WC, Waist Circumference; FBG, Fasting Blood Glucose; HbA1c, Glycated Hemoglobin; Ca, Calcium; Phos, Phosphorus; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; TG, Triglyceride; TC, Total Cholesterol; T2DM, Type 2 Diabetes Mellitus; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure

<0.001), TG (P-value = 0.001), SBP (P-value = 0.048), DBP (P-value = 0.041) and 25(OH)D serum levels (P-value = 0.018) were significantly different between cases and controls (*Table II*).

We found significant differences in allelic (A) and genotypic (CA) frequencies for the rs4588 polymorphism (P = 0.028, OR = 1.491, 95% CI = 1.043–2.132 and P = 0.0032, OR = 2.578, 95% CI = 1.579–4.208 respectively) and the GG genotype for rs7041 (P = 0.029, OR = 0.537, 95% CI = 0.306–0.944) (*Table III*). Further, we analyzed the association of rs4588 genotypes with CAD under four genetic models (co-dominant, dominant, recessive and over-dominant) to choose the best fitted model. The AIC (Akaike's Information Criterion) was calculated. The fitted model of inheritance with the lowest AIC value was over-dominant and dominant for rs4588 and rs7041, respectively.

Moreover, we observed that CA/GG, CA/GT, and CA/TT diplotypes were remarkably linked with CAD. *Table V* shows that there were no significant associations between demographic and clinical characteristics, with the genotypes of rs7041 and rs4588 polymorphisms.

## Discussion

In this case-control study, we observed that rs4588 A-allele and CA-genotype, and rs 7041 GG-genotype were associated with CAD susceptibility. Moreover, circulating levels of 25(OH)D were significantly lower in CAD patients as compared to healthy controls. However, there was no association between the circulating levels of 25(OH)D and genotypes of rs4588/rs7041. Low circulating levels of 25(OH)D have been linked with the increased risk of CAD, and this relation may differ in various populations (9–17). Ethnic variations in VDBP polymorphisms might be responsible for the altered bioavailability (25, 28). Several investigations have been conducted to explore the associations of vitamin D with the risk of CAD; however, we still do not know clearly whether VDBP plays a role in the CAD pathogenesis.

The association of rs7041 and rs4588 polymorphisms of the VDBP gene and the risk of several diseases has been the subject of several investigations but the results have been controversial (35–38). VDBP is the principal factor in determining serum vitamin D concentration, and therefore it has been considered as a critical factor for CAD pathogenesis.

According to our results, rs4588 A-allele and CA-genotype were associated with CAD. On the con-

**Table III** Allelic and genotypic frequencies of rs7041 and rs4588 polymorphisms in CAD patients and control group.

| Alleles/Genotypes | | Models | CAD N (%) | Control N (%) | OR (95% CI) | p-value* | AIC |
|---|---|---|---|---|---|---|---|
| rs4588 | C | allelic | 187 (65.4%) | 214 (73.8%) | Referent | 0.028 | |
| | A | | 99 (34.6%) | 70 (26.2%) | 1.491 (1.043–2.132) | | |
| | CC | Co-dominant | 50 (35.0%) | 80 (55.2%) | Referent | 0.00032 | 389 |
| | CA | | 87 (60.8%) | 54 (37.2%) | 2.578 (1.579–4.208) | | |
| | AA | | 6 (4.2%) | 11 (7.6%) | 0.873 (0.535–1.425) | | |
| | CA+AA | Dominant | 93 (65%) | 65 (44.8%) | Referent | 0.001 | 391.3 |
| | CC | | 50 (35%) | 80 (55.2%) | 0.437 (0.272–0.702) | | |
| | CC+CA | Recessive | 137 (95.8%) | 134 (92.4%) | Referent | 0.222 | 401.7 |
| | AA | | 6 (4.2%) | 11 (7.6%) | 0.534 (0.192–1.484) | | |
| | CC+AA | Over-dominant | 56 (39.2%) | 91 (62.8%) | Referent | <0.001 | 387 |
| | CA | | 87 (60.8%) | 54 (37.2%) | 2.617 (1.626–4.219) | | |
| HWE | | | | | | 0.655 | |
| rs7041 | T | allelic | 150 (52.4%) | 137 (47.2%) | 0.812 (0.585–1.126) | 0.211 | |
| | G | | 136 (47.6%) | 153 (52.8%) | Referent | | |
| | TT | Co-dominant | 32 (22.4%) | 33 (22.8%) | 1.590 (0.891–2.837) | 0.070 | 399.9 |
| | TG | | 86 (60.1%) | 71 (49.0%) | 1.986 (1.113–3.544) | | |
| | GG | | 25 (17.5%) | 41 (28.3%) | Referent | | |
| | TT+TG | Dominant | 118 (82.5%) | 104 (71.7%) | Referent | 0.029 | 398.5 |
| | GG | | 25 (17.5%) | 41 (28.3%) | 0.537 (0.306–0.944) | | |
| | TG+GG | Recessive | 111 (77.6) | 112 (77.2) | Referent | 0.938 | 403.2 |
| | TT | | 32 (22.4) | 33 (22.8) | 0.978 (0.563–1.701) | | |
| | TT+GG | Over-dominant | 57 (39.9%) | 74 (51%) | Referent | 0.057 | 399.6 |
| | TG | | 86 (60.1%) | 71 (49%) | 1.572 (0.986–2.506) | | |
| HWE | | | | | | 0.831 | |
| | | * Chi-square test; HWE, Hardy-Weinberg Equilibrium test p-value<br><br>CAD, Coronary Artery Disease; OR, Odds Ratio; CI, Confidence Interval, AIC, Akaike Information Criterion | | | | | |

**Table IV** Diplotype frequencies of rs4588 and rs7041 polymorphisms in CAD patients and control group.

| rs4588/rs7041 diplotype | CAD | Control | p-value | OR (95% CI) |
|---|---|---|---|---|
| CC/GG | 18 (11.3%) | 40 (27.6%) | 1 | Referent |
| AA/TT | 3 (1.9%) | 11 (7.6%) | 0.478 | 0.606 (0.151–2.439) |
| CA/GG | 7 (4.4%) | 1 (0.7%) | 0.002 | 15.556 (1.780–135.956) |
| CA/TG | 57 (35.8%) | 40 (27.6%) | 0.001 | 3.167 (1.592–6.299) |
| CA/TT | 23 (14.5%) | 13 (9%) | 0.002 | 3.932 (1.633–9.466) |
| CC/TG | 26 (16.4%) | 31 (21.4%) | 0.108 | 1.864 (0.870–3.995) |
| CC/TT | 6 (3.8%) | 9 (6.2%) | 0.510 | 1.481 (0.458–4.789) |
| CAD, Coronary Artery Disease; OR, Odds Ratio; CI, Confidence Interval | | | | |

**Table V** Association of demographic data with genotypic frequencies for rs4588 and rs7041 polymorphisms.

| Characte-ristics | rs4588 | | | Kruskal-Wallis | rs7041 | | | Kruskal-Wallis |
|---|---|---|---|---|---|---|---|---|
| | CC | CA | AA | | TT | TG | GG | |
| | Median (IQR) | Median (IQR) | Median (IQR) | p-value | Median (IQR) | Median (IQR) | Median (IQR) | p-value |
| Age (yr) | 54 (50–59) | 57 (52–64) | 58 (52–66) | 0.046 | 58 (52–65) | 56 (51–63) | 57 (51–60) | 0.235 |
| BMI (kg/m²) | 26 (24–29) | 26 (24–29) | 26 (24–26) | 0.482 | 26 (24–28) | 26 (24–29) | 27 (25–29) | 0.350 |
| WC (cm) | 97 (91–105) | 96 (91–102) | 92 (88–96) | 0.209 | 95 (90–99) | 96 (90–103) | 100 (93–105) | 0.375 |
| FBG (mmol/L) | 5.10 (4.71–5.49) | 5.10 (4.665.49) | 4.88 (85–97) | 0.706 | 5.05 (4.77–5.55) | 5.10 (4.66–5.43) | 5.16 (4.71–5.55) | 0.713 |
| LDL-C (mmol/L) | 2.35 (1.96–2.87) | 2.46 (1.86–3.21) | 2.40 (1.89–3.15) | 0.662 | 2.66 (2.14–3.28) | 2.30 (1.83–2.95) | 2.35 (2.02–2.79) | 0.093 |
| HDL-C (mmol/L) | 0.98 (0.80–1.16) | 0.90 (0.77–1.03) | 1.06 (0.90–1.13) | 0.082 | 0.93 (0.80–1.11) | 0.95 (0.77–1.13) | 0.93 (0.75–1.06) | 0.820 |
| TC (mmol/L) | 3.85 (3.28–4.61) | 4.01 (3.18–4.92) | 4.09 (3.18–4.92) | 0.621 | 4.14 (3.44–4.97) | 3.78 (3.13–4.79) | 4.09 (3.49–4.66) | 0.075 |
| TG (mmol/L) | 1.33 (1.02–1.92) | 1.41 (0.97–2.12) | 1.29 (0.83–2.09) | 0.624 | 1.41 (0.96–2.14) | 1.38 (1–1.94) | 1.29 (0.99–2.02) | 0.920 |
| Ca (mmol/L) | 2.25 (2.25–2.50) | 2.25 (2.25–2.25) | 2.25 (2.25–2.25) | 0.205 | 2.25 (2.25–2.50) | 2.25 (2.25–2.50) | 2.25 (2.25–2.25) | 0.154 |
| Phos (mmol/L) | 0.95 (0.83–1.11) | 0.96 (0.84–1.13) | 1.01 (0.85–1.27) | 0.381 | 0.95 (0.85–1.09) | 0.98 (0.82–1.14) | 0.93 (0.87–1.11) | 0.253 |
| 25(OH)D (nmol/L) | 26.44 (19.56–42.67) | 27.29 (16.28–41.11) | 22.94 (21.31–58.52) | 0.816 | 24.53 (16.43–36.89) | 26.62 (17.79–39.36) | 32.92 (17.59–55.53) | 0.233 |
| SBP (mmHg) | 26.44 (19.56–42.67) | 120 (110–130) | 120 (115–121.5) | 0.997 | 120 (110–120) | 120 (110–130) | 120 (110–130) | 0.386 |
| DBP (mmHg) | 73 (70–80) | 78 (70–80) | 80 (75–80) | 0.491 | 80 (70–80) | 80 (70–80) | 70 (60–73) | 0.070 |
| Continuous variables are presented as median (interquartile range: P25-P75). Differences between cases and controls are obtained based on the Mann-Whitney U test. CAD, Coronary Artery Disease; BMI, Body Mass Index; WC, Waist Circumference FBG, Fasting Blood Glucose; HbA1c, Glycated Hemoglobin; Ca, Calcium; Phos, Phosphorus; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; TG, Triglyceride; TC, Total Cholesterol; T2DM, Type 2 Diabetes Mellitus; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure | | | | | | | | |

trary, Michos et al. (39) found that rs4588 had no effect on coronary heart disease (CHD) incidence in both black and white races of USA in the AIRC (Atherosclerosis Risk in Communities) study (40). Nissen et al. (41) mentioned that the A-allele of rs4588 was significantly associated with lower serum 25(OH)D concentrations. However, we did not find such links between 25(OH)D circulating levels and rs4588. These discrepancies might be due to the ethnic-based variations in communities. However, Maneechay et al. (42) reported that rs4588 affects 25(OH)D levels. This study revealed an association between the A allele of rs4588 and cancer in men under 60 years of age suffering from colorectal cancer, which is similar to our findings.

We observed significant differences between cases and controls regarding the frequency of rs7041 genotypes. Our findings are in contrast with the Michos et al. study. They showed that there was no main effect of rs7041 on CHD incidence in both white and black races of USA in the AIRC study (42). Moreover, Jorde et al. found no association between CVD risk factors and rs7041 or other GC gene SNPs (43). However, Pamela et al. (39) have revealed that the risk of heart failure (HF) is increased by low circulating levels of 25(OH)D and the rs7041 G allele. These inconsistencies might be explained based on the different pathomechanisms of CAD and HF, sample size and ethnic diversity.

In contrast to several studies, we did not find any significant differences regarding the circulating levels of 25(OH)D among various VDBP genotypes (44–46). However, Lafi et al. (47) reported that the genotypes TT and TG of rs7041 and AA and AC of rs4588 were linked with lower 25(OH)D levels in healthy Jordanian people. Moreover, in unhealthy

patients, merely a variant allele of rs7041 (TT) was associated with higher 25(OH)D levels (47). Similar to our findings, Almesri et al. (48) reported that rs7041 minor allele (G) and rare genotype (GG) did not influence 25(OH)D levels. However, a minor allele of rs4588 (C) was associated with low 25(OH)D plasma levels.

One limitation of this study is that we could not determine the VDBP circulating levels and gene expression. However, our recruited homogeneous samples of Iranian men with confirmed CAD could enable us to make reliable conclusions about the association of VDBP genotypes with CAD.

In conclusion, VDBP polymorphisms affect the susceptibility to CAD among Iranian men. Therefore, further studies are suggested for the association of VDBP phenotypes and its serum levels with CAD. Also, based on the available data reported here, it seems that different populations show different results according to their various forms of these SNPs and there is still a need for further investigation in our region to obtain better explanations.

## Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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